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# Polymers for Fluorescence Imaging of Formaldehyde in Living Systems via the Hantzsch Reaction

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**ABSTRACT:** Formaldehyde (FA) has been detected via the Hantzsch reaction for many decades. However, the Hantzsch reaction has been rarely used to detect FA in biological systems due to the disadvantages of small-molecule probes (including toxicity and poor water solubility). In this study, polymeric fluorescent probes were developed to resolve these issues associated with small molecules, and FA in living systems was successfully detected via the Hantzsch reaction. These water-soluble polymers were easily scaled-up (~25 g) by radical polymerization using commercial monomers. These polymers exhibited similar, albeit better, sensitivity to FA compared to water-soluble small molecules, primarily indicative of the advantages of polymers for the detection of FA via the Hantzsch reaction. The polymer structures were highly biocompatible with the probes; thus, these polymers can effectively detect endogenous FA in cells or zebrafish in a safe manner. This result confirmed the superiority of polymers in safety as biomaterials. This study highlights a straightforward method for exploring new probes for the detection of FA in living systems. To the best of our knowledge, this is the first study reporting the detection of FA in biological systems via the Hantzsch reaction. It offers new functional polymers for bioimaging and extends the application scope of the Hantzsch reaction, reflecting the utility of a broad study of organic reactions in interdisciplinary fields as well as possible key implications in organic chemistry, analytical chemistry, and polymer chemistry.

## INTRODUCTION

Formaldehyde (FA) is a well-known environmental pollutant and human toxin. FA cross-links DNA or proteins, and high FA concentrations can lead to stomach ache, emesis, heart disorders, renal injury, and even death.<sup>1-5</sup> Conversely, FA is endogenously generated in the human body as an essential metabolite in several biochemical processes (including the epigenetic *N*-demethylation of DNA and/or RNA; small molecular metabolism; the action of the neutrophil enzyme myeloperoxidase; and oxidase enzymes).<sup>6-11</sup> The concentrations of FA in human body are controlled at stable levels (50–100  $\mu\text{M}$  in blood and 200–500  $\mu\text{M}$  in cells) via compensatory FA metabolism by FA dehydrogenase. Higher FA concentrations are considered to be related to Alzheimer's disease; cancer; deformed embryo, and respiratory disorders.<sup>4, 9, 12-15</sup> Therefore, it is important to detect FA in biological systems to investigate and monitor diseases in terms of fundamental studies and practical applications.

Conventionally, FA can be detected by high-performance liquid chromatography, mass spectrometry, gas chromatography, and preconcentration or chemical ionization,<sup>16-21</sup> all of which exhibit high sensitivity and selectivity; nevertheless, these methods are not suitable for detecting FA in living organisms due to the inevitable destruction of specimens and tedious sample preparation procedures. Meanwhile, fluorescent probes constitute a straightforward method for the nondestructive ex-

amination of FA in living systems. In 2015, Chang and coworkers were the first to design a fluorescent probe via an FA-induced 2-aza-Cope reaction, which was used to successfully detect exogenous or endogenous FA in cells.<sup>22-23</sup> Chang and Lin have developed several sensitive fluorescent FA probes via the 2-aza-Cope rearrangement or hydrazine-aldehyde condensation reactions.<sup>24-27</sup> These studies have opened new avenues for the detection of FA in living organisms in a facile manner and have triggered the development of a series of small-molecule probes for FA detection.<sup>14, 28-31</sup>

Inspired by such pioneering studies, a polymeric fluorescent probe for detecting FA in living systems via the Hantzsch reaction is reported for the first time (Scheme 1). The Hantzsch reaction, as introduced by Hantzsch in 1881,<sup>32</sup> involves the one-pot condensation of an aldehyde, two  $\beta$ -diketone or  $\beta$ -ketone ester derivatives, and ammonia, efficiently yielding 1,4-dihydropyridine (1,4-DHP) with native fluorescence (Scheme 1a). Several 1,4-DHP derivatives have been developed via the Hantzsch reaction for the treatment of hypertension and other cardiovascular diseases.<sup>33-36</sup> Notably, the Hantzsch reaction has also been used for FA detection because of its rapid reaction rate and native fluorescence of 1,4-DHP. In 1953, Nash reported the first colorimetric estimation of FA via the Hantzsch reaction.<sup>37</sup> Thus far, several approaches including commercial FA assay kits have been successfully developed on the basis of the Hantzsch reaction to detect FA in the environment, food,

and medicine.<sup>38–40</sup> Nevertheless, the Hantzsch reaction has been rarely used to detect FA in living organisms to the best of our knowledge, possibly related to the clear toxicity or poor water solubility of small-molecule  $\beta$ -diketone or  $\beta$ -ketone ester derivatives.

**Scheme 1. a) The Hantzsch reaction, and b) A polymer for the detection of FA in living systems via the Hantzsch reaction.**



In this study, polymeric probes were developed to overcome the inherent limitations of small molecules. In this regard, water-soluble copolymers containing  $\beta$ -ketone ester moieties were easily prepared by radical polymerization. These copolymers exhibited similar, albeit better, sensitivity compared to small molecules (such as acetylacetone (Hacac) and ethyl acetoacetate (Eaa)) to detect FA via the Hantzsch reaction; these polymers also exhibited excellent cellular safety. A biocompatible polymer was used to successfully detect endogenous FA in living systems (including cells and zebrafish, Scheme 1b). This study highlights the utility of combining polymer chemistry and organic reactions to explore new biomaterials for practical applications.

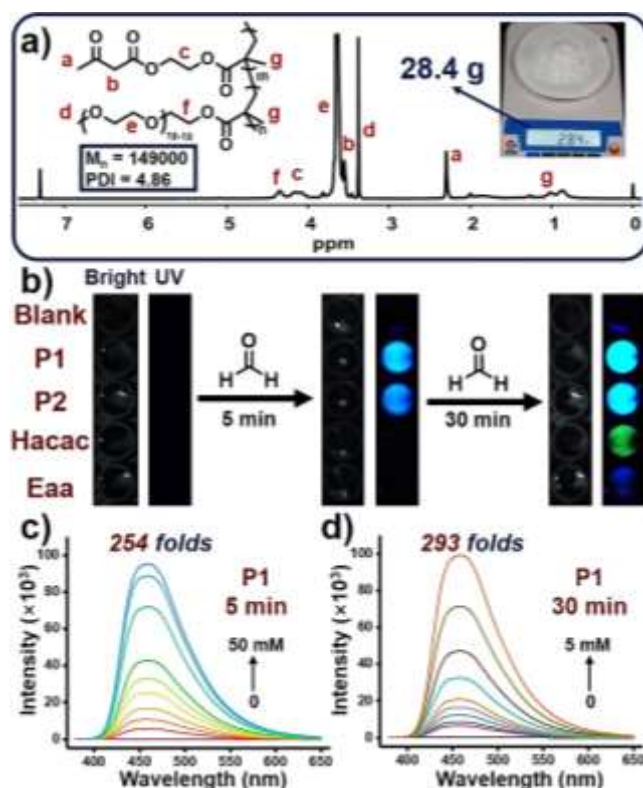
## RESULTS AND DISCUSSION

### Comparison of Polymers to Small Molecules for the Detection of FA

$\beta$ -Ketone-ester-containing polymers were prepared using commercially available monomers by convenient conventional radical polymerization. As a typical example, two commercial monomers, i.e., 2-(acetoacetoxy) ethyl methacrylate (AEMA) and poly(ethylene glycol methyl ether) methacrylate (PEGMA,  $M_n$ :  $\sim 950$  g mol<sup>-1</sup>), respectively, were copolymerized by radical polymerization. The AEMA:PEGMA molar ratio was set to be 1:1. 2,2'-Azobis(2,4-dimethylvaleronitrile) (ABVN, 2 mol% to monomers) and DMF were used as the initiator and solvent, respectively. First, a Schlenk tube was charged with the monomers, initiator, and solvent, followed by purging the tube with nitrogen for 30 min to remove oxygen and maintaining the tube in a 70°C oil bath. After 12 h, polymerization was quenched in an ice-water bath, and the samples were subjected to <sup>1</sup>H NMR and gel permeation chromatography (GPC) analyses. The final copolymer was obtained after precipitation in diethyl ether, referred to as P1. Monomers were nearly completely polymerized ( $\sim 99\%$ ), yielding P1,  $\sim 28$  g (Figure 1a) with a satisfactory molecular weight ( $M_n$ (GPC):  $\sim 149000$  g mol<sup>-1</sup>, Table S1, Figure 1a). The <sup>1</sup>H NMR spectrum of P1 (Figure 1a) revealed clear peaks at  $\sim 3.33$  ppm, corresponding to the specific methyl

groups in PEG segments, and at  $\sim 2.25$  ppm, corresponding to  $\beta$ -ketone ester. An integral ratio ( $I_{3.33}/I_{2.25}$ ) of 1.01 was consistent with the theoretical value (1:1), indicative of the successful preparation of the target poly( $\beta$ -ketone ester)-co-poly(PEGMA). In addition, another polymer (P2) was prepared using a different AEMA/PEGMA feed ratio (1/2) by a similar procedure (Table S1, Figure S1).

The resulting copolymers were used for the qualitative detection of FA via the Hantzsch reaction (Figure 1b). In a 96-well plate, a PBS solution (pH = 7.4) of P1 (10 mg/mL,  $\beta$ -ketone ester: 8.6 mM) and ammonium acetate (1.0 mg/mL) was incubated with FA (5 mM) for 5 min (20°C), followed by subjecting the plate for UV observation. P2 (10 mg/mL,  $\beta$ -ketone ester: 4.7 mM) was analyzed in a similar manner. Small molecules including 8.6 mM of Hacac and 8.6 mM of Eaa were used as the controls, and PBS served as the blank.



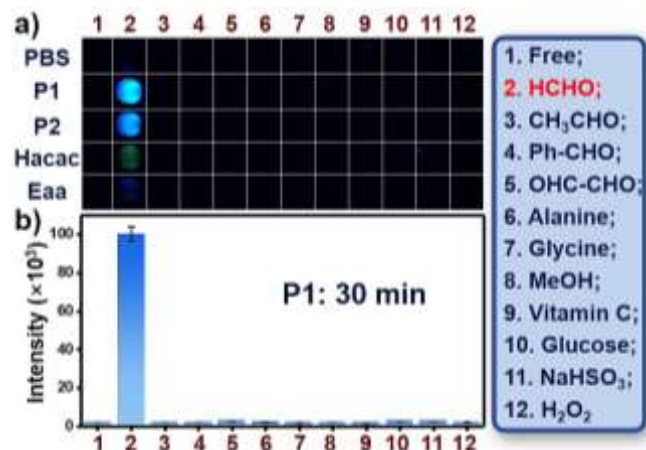
**Figure 1.** a) <sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>) of P1. b) Optical images of copolymers (10 mg/mL;  $\beta$ -ketone ester: P1 (8.6 mM), P2 (4.7 mM)) and small molecules (Hacac: 8.6 mM, Eaa: 8.6 mM) for reaction with FA (5 mM) in the presence of ammonium acetate (1.0 mg/mL), 20°C. PBS (pH 7.4) was used as the solvent and blank. c) Fluorescence spectra of the 5-min treatment of P1 (10 mg/mL,  $\beta$ -ketone ester: 8.6 mM) and ammonium acetate (1.0 mg/mL) with FA (0–50 mM) in PBS (pH 7.4), 20°C. d) Fluorescence spectra of 30-min treatment of P1 (10 mg/mL,  $\beta$ -ketone ester: 8.6 mM) and ammonium acetate (1.0 mg/mL) with FA (0–5 mM) in PBS (pH 7.4), 20°C.

None of the solutions exhibited fluorescence prior to the addition of FA (Figure 1b, left). However, after 5-min incubation with FA, P1 and P2 solutions exhibited strong fluorescence. P1 exhibited a more intense fluorescence than P2, related to the presence of higher amounts of  $\beta$ -ketone ester in the P1 structure. In contrast, the Hacac and Eaa solutions did not exhibit any fluorescence (Figure 1b, middle). After 30 min, the small molecule solutions also exhibited visible, albeit weak, fluorescence, while the fluorescence strength of the polymer solutions increased. This suggested the more rapid turn-on response of pol-

ymers compared to small molecules, possibly related to the acceleration of the concentration-dependent Hantzsch reaction by higher concentrations of  $\beta$ -ketone ester in the polymer structures. Subsequently, the mixtures in the plate were extracted by  $\text{CDCl}_3$  for  $^1\text{H}$  NMR analysis, and 1,4-DHP was identified, indicative of the facile Hantzsch reaction in the microplate (Figure S2).

In addition, the absorption profiles of polymers and small molecules were examined. Intense fluorescence peaks ( $\sim 460$  nm) were observed after the 5-min incubation of FA (0–50 mM) in a solution of P1 (10 mg/mL,  $\beta$ -ketone ester: 8.6 mM) and ammonium acetate (1.0 mg/mL) (20°C), with a 254-fold fluorescence enhancement (Figure 1c). After incubation with 5 mM of FA for 30 min, a 293-fold fluorescence increase was recorded (Figure 1d). Similar results were obtained for the P2 solution (5 min: 91-fold fluorescence enhancement, 30 min: 110-fold fluorescence enhancement;  $\sim 460$  nm, Figure S3a and 3a', respectively). However, Hacac only exhibited 37-fold and 69-fold fluorescence enhancement ( $\sim 510$  nm) in the 5- and 30-min tests (Figure S3b and 3b', respectively). Eaa exhibited 21-fold and 101-fold fluorescence enhancement in the 5- and 30-min tests (Figure S3c and 3c', respectively). The calculated reaction rate constants of probes (Figure S4) followed the order of P1 ( $0.072 \text{ min}^{-1}$ ) > P2 ( $0.069 \text{ min}^{-1}$ ) > Hacac ( $0.064 \text{ min}^{-1}$ ) > Eaa ( $0.040 \text{ min}^{-1}$ ). This result confirmed that polymers are more rapid turn-on probes compared to small molecules for FA detection. Meanwhile, the calculated FA detection thresholds for P1, P2, Hacac, and Eaa were  $3.1 \times 10^{-7} \text{ M}$ ,  $3.4 \times 10^{-7} \text{ M}$ ,  $1.8 \times 10^{-6} \text{ M}$ , and  $1.2 \times 10^{-6} \text{ M}$ , respectively, on the basis of titration results (Figure S5). This result suggested that polymers are more sensitive than small molecules for the detection of FA.

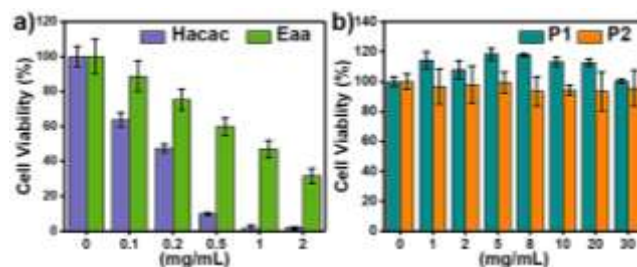
Furthermore, P1, P2, Hacac, and Eaa exhibited higher selectivity (5-min and 30-min tests) for FA compared to other biological species. The fluorescence intensities of polymers were greater than 10 times those of small molecules (Figures 2 and S6). Thus, these copolymers can serve as better probes than small molecules for the detection of FA via the Hantzsch reaction.



**Figure 2.** Selectivity study of polymers (10 mg/mL) and small molecules (8.6 mM) to FA (5 mM) and other biological species (5 mM) in the presence of ammonium acetate (1.0 mg/mL), 20°C, 30 min. a) Photos of probes with FA and other species, PBS (pH 7.4),  $\lambda_{\text{ex}} = 360$  nm. b) Fluorescent intensities of P1 (10 mg/mL) and ammonium acetate (1 mg/mL) to FA (5 mM) and other biological species (5 mM) ( $p < 0.01$  between FA and other species). Data are represented as mean  $\pm$  standard deviation (SD).

## Cytotoxicity Study of Small Molecules and Polymers

Fluorescent probes should always be biocompatible for use in biological systems. Thus, the cytotoxicity of P1, P2, Hacac, and Eaa was evaluated using a cell-counting kit-8 (CCK-8) assay. The murine fibroblast cell line L929 was used as the model cell, where cell viability in the medium only was defined as 100% viability.



**Figure 3.** Cytotoxicity of a) small molecules (Hacac and Eaa) and b) polymers to L929 cells, 24-h culture. Results are represented as mean  $\pm$  standard deviation (SD).

Hacac exhibited clear cytotoxicity, i.e.,  $\sim 64\%$  and  $\sim 47\%$  viability at 0.1 mg/mL and 0.2 mg/mL, respectively (Figure 3a,  $p < 0.01$ , contrast with cells in medium only). The half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) value of Hacac was calculated to be 0.16 mg/mL by SPSS 15.0. Eaa exhibited moderate cytotoxicity to L929 cells (Figure 3a), and the calculated  $\text{IC}_{50}$  value of Eaa was 0.95 mg/mL. On the contrary, cells retained nearly 100% viability even in 30 mg/mL of P1 ( $\beta$ -ketone ester: 5.5 mg/mL) or P2 ( $\beta$ -ketone ester: 3.0 mg/mL) (Figure 3b). This result suggested the unique advantage of polymers to serve as safe biomaterials even with the inclusion of some toxic groups in the polymer structure.

## Detection of Exogenous FA in Cells

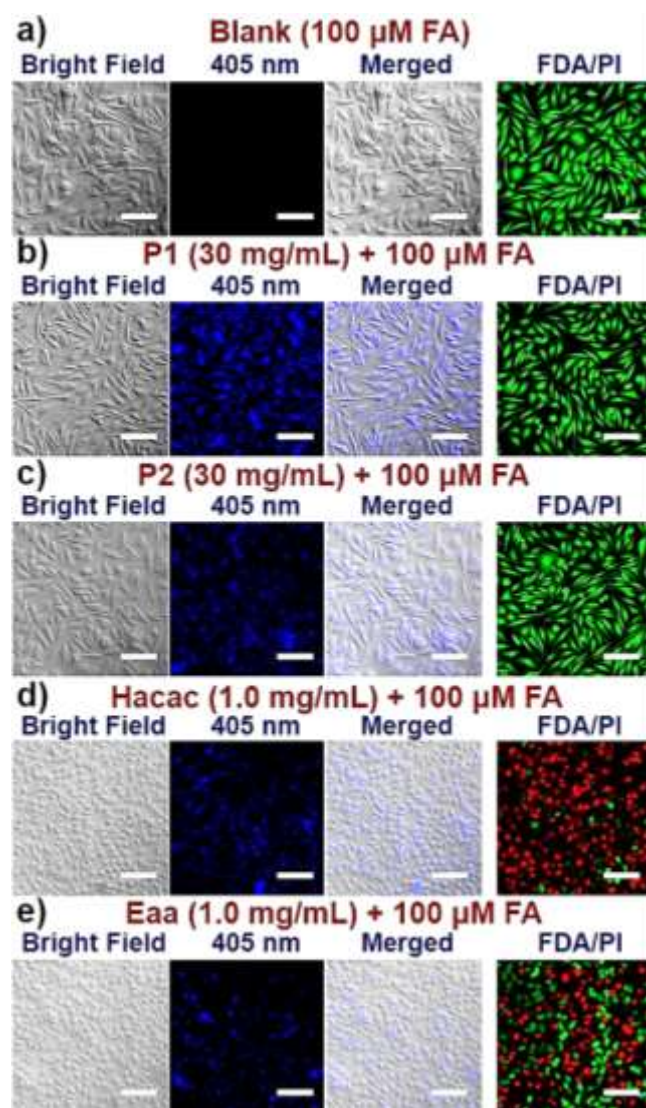
Polymers and small molecules were used to detect the excess added FA in L929 cells. Typically, cells ( $\sim 10^5/\text{mL}$ ) were incubated in a culture medium containing P1 (30 mg/mL) and ammonium acetate (1 mg/mL) for 1 h, followed by washing thrice with PBS (pH = 7.4) and incubating in a culture medium containing FA (100  $\mu\text{M}$ ) for 30 min prior to observation. Cells without probes served as the blank.

In the presence of FA, cells exhibited fluorescence by using P1, indicative of the smooth Hantzsch reaction (Figure 4b). By using P2 (30 mg/mL), cells also exhibited clear, albeit weaker, fluorescence (Figure 4c). No fluorescence and extremely weak fluorescence were recorded with 0.1 and 0.5 mg/mL of small molecules, respectively (Figure S7). Visible fluorescence was only observed with higher concentration (1 mg/mL) of Hacac or Eaa (Figure 4d and 4e). These visible results are in good agreement with the quantitative fluorescence intensity data (Figure S8a) and flow cytometric analysis (Figure S8b), suggesting that the Hantzsch reaction is a simple, practical tool for tracing the additional FA in living cells.

In addition, the fluorescein diacetate (FDA)/propidium iodide (PI) assay<sup>41</sup> was employed to simultaneously observe living or dead cells after FA detection. Cells exhibited nearly 100% viability in the presence of polymers (Figures 4b, 4c, and S9). Few cells survived 1.0 mg/mL of Hacac (Figures 4d and S9), and remarkable apoptosis was observed in the presence of 1.0 mg/mL of Eaa (Figures 4e and S9). These results are consistent with those obtained from quantitative CCK-8 analyses (Figure S10), indicative of the superiority of polymers to detect FA in a



safe manner, thereby ensuring the accuracy of the detection results.



**Figure 4.** Exogenous detection of FA in L929 cells. a) Blank (without probes); b) P1; c) P2; d) Hacac; and e) Eaa. Cells were incubated with or without probes in the presence of ammonium acetate (1.0 mg/mL) for 1 h, followed by incubation with FA (100  $\mu$ M) for 30 min. Bar = 100  $\mu$ m.

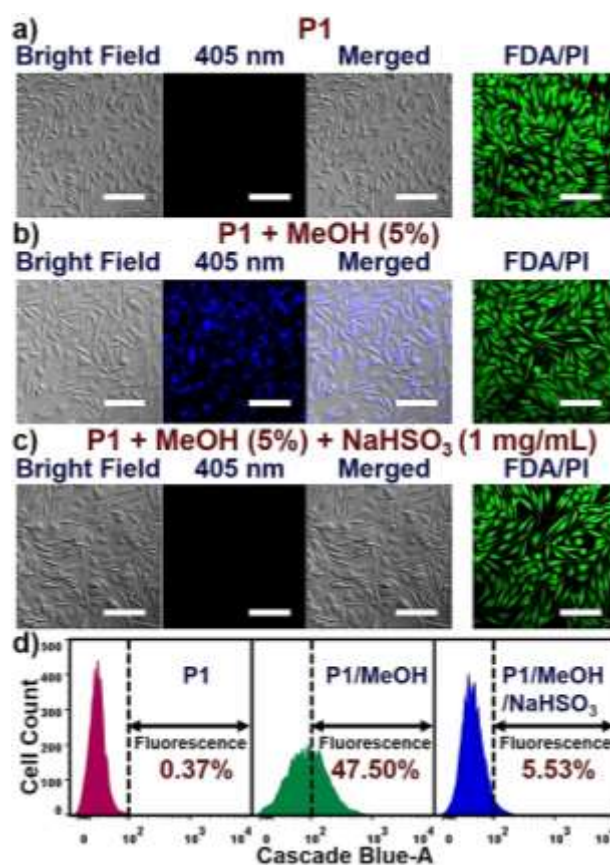
Moreover, the subcellular distribution of polymers (e.g., P1) was studied via the reported methods.<sup>22-24</sup> L929 cells ( $\sim 10^5$ /mL) were incubated with P1 (30 mg/mL), ammonium acetate (1 mg/mL), and an indicator for 1 h, followed by culturing with FA (100  $\mu$ M) for 30 min. Four indicators were analyzed in parallel (i.e., LysoTracker Red, ER-Tracker Red, Golgi-Tracker Red, and MitoTracker Green, respectively). Co-localization data suggested that the Hantzsch reaction mainly occurs in the endoplasmic reticulum and Golgi apparatus (Figure S11). This result is different from that reported previously,<sup>22-24</sup> possibly related to different endocytosis and cellular metabolism between polymers and small molecules.

These results confirmed that the obtained polymers are biocompatible probes for the detection of FA. Thus, P1 is selected for the detection of endogenous FA in living cells; it exhibits excellent safety and FA detection ability.

#### Detection of Methanol-Induced Endogenous FA in Cells

Methanol was used to induce endogenous FA in cells.  $\text{NaHSO}_3$  has been reported to be an FA inhibitor.<sup>24, 27</sup> L929 cells ( $\sim 10^5$ /mL) were incubated in a culture medium containing methanol (5 vol%), P1 (30 mg/mL), and ammonium acetate (1 mg/mL) for 1 h prior to observation. Cells in methanol (5 vol%), P1 (30 mg/mL), ammonium acetate (1 mg/mL), and  $\text{NaHSO}_3$  (1 mg/mL) were examined as the control. Cells in the culture medium containing P1 (30 mg/mL) and ammonium acetate (1 mg/mL) served as the blank.

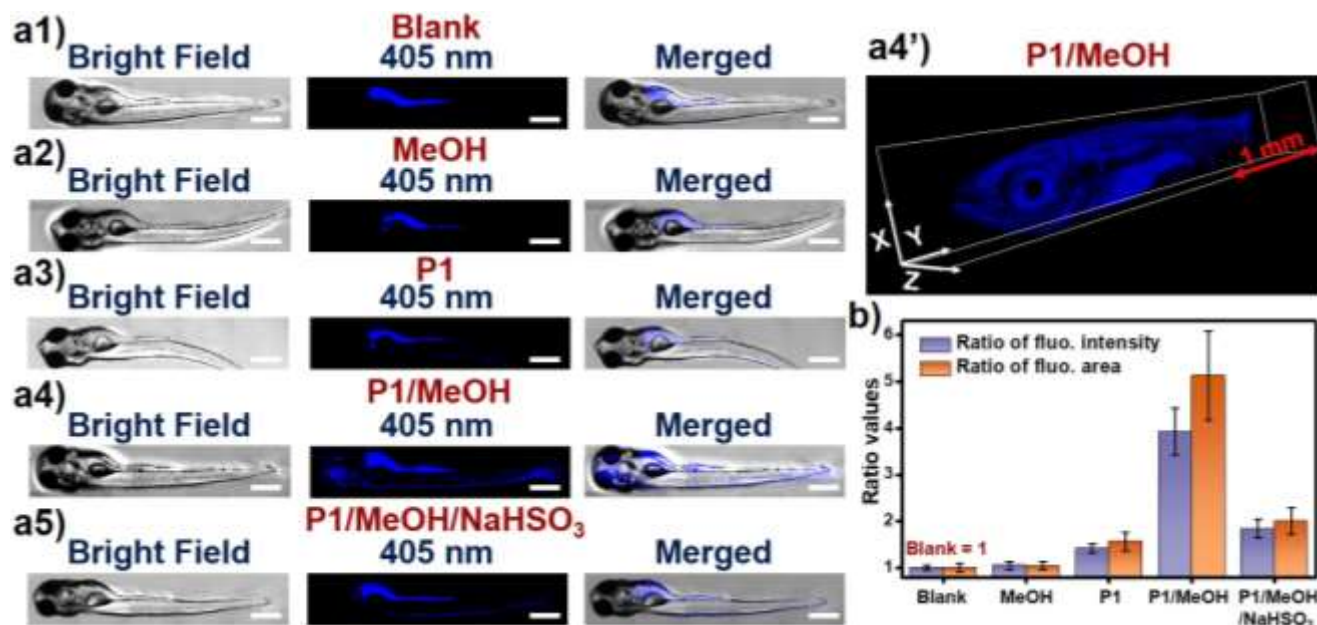
Cells exhibited clear fluorescence (Figure 5b), while the blank and control did not exhibit fluorescence (Figure 5a and 5c). The direct observation results agreed with the quantitative data obtained by flow cytometric analysis (Figure 5d), confirming that FA in cells as a metabolite of methanol is effectively detected by P1 or inhibited by  $\text{NaHSO}_3$ . Meanwhile, cells retained  $\sim 100\%$  viability after the experiments (Figures 5 and S12a: FDA/PI assay; Figure S12b: CCK-8 assay), suggesting that P1 is a valid, safe probe to detect endogenous FA in living cells.



**Figure 5.** Detection of methanol-induced endogenous FA in L929 cells. a) P1; b) P1 + methanol; c) P1 + methanol +  $\text{NaHSO}_3$ . Cells were incubated in the presence of ammonium acetate (1 mg/mL), 1 h, bar = 100  $\mu$ m; d) flow cytometry analysis of fluorescent cells. Cascade Blue-A channel: excitation wavelength-405 nm.

#### Detection of Methanol-Induced Endogenous FA in Zebrafish

Moreover, P1 was used to detect endogenous FA in zebrafish. Six zebrafish larvae ( $\sim 15$  days old) were cultured in water ( $\sim 20$  mL) containing methanol (5 vol%), P1 (30 mg/mL), and ammonium acetate (1 mg/mL) for 2 h. Then, the larvae were paralyzed in a tricaine solution (4  $\mu$ g/mL,  $\sim 10$  min) prior to observation. Larvae cultured under different conditions were



**Figure 6.** Images of zebrafish in a1) water (blank); a2) methanol (5 vol%); a3) P1 (30 mg/mL) + ammonium acetate (1 mg/mL); a4, a4') P1 (30 mg/mL) + ammonium acetate (1 mg/mL) + methanol (5 vol%); a5) P1 (30 mg/mL) + ammonium acetate (1 mg/mL) + methanol (5 vol%) + NaHSO<sub>3</sub> (1 mg/mL). b) Fluorescent intensity ratio and fluorescent area ratio of fish in different groups, ratios in the blank were defined as 1. Data are represented as mean  $\pm$  standard deviation (SD).

tested as controls in a similar manner. These conditions were as follows: methanol (5 vol%); P1 (30 mg/mL) + ammonium acetate (1 mg/mL); and methanol (5 vol%) + NaHSO<sub>3</sub> (1 mg/mL) + P1 (30 mg/mL) + ammonium acetate (1 mg/mL). Larvae in water only served as the blank.

A fluorescence signal was observed for the internal parts of the blank larvae (Figure 6a1). When the larvae were incubated with methanol, almost the same images as the blank were observed (Figure 6a2). After incubation with P1, significant fluorescence enhancement was not observed (Figure 6a3). Quantitative data indicated a slight increase in the fluorescent strength and area of larvae (Figure 6b,  $p < 0.05$ , contrast to the blank), corresponding to the marginal amount of intrinsic FA in the larvae. However, in the presence of methanol and P1, the head and tail of larvae exhibited clear fluorescence (Figure 6a4). Further 3D images obtained from a light sheet fluorescent microscopy revealed that the head, back, and backbone of the zebrafish larvae in methanol and P1 exhibit stronger fluorescence compared with those under other conditions (Figures 6a4' and S13). Hence, the fluorescence intensity and area of larvae incubated in methanol and P1 significantly increase (Figure 6b,  $p < 0.01$ , contrast to the blank). This result confirmed that the use of methanol is effective for inducing endogenous FA in zebrafish larvae, and P1 is suitable for detecting FA in living systems.

With the addition of NaHSO<sub>3</sub>, larvae exhibited a remarkably decreased fluorescence signal (Figures 6a5, 6b, and S13), confirming that NaHSO<sub>3</sub> is a simple, albeit efficient, FA inhibitor to remove FA in living systems.

#### Possible Extensions and Challenges

With the assistance of polymers, the detection of endogenous FA in living systems was initially realized by the Hantzsch reaction; however, current research still has room for improvement.

The polymers in this study were only suitable for the detection of FA in cells or transparent small animals because of the blue fluorescent Hantzsch product. To use the polymeric probes in other animals or possibly clinical trials, it is crucial to exploit polymers containing new  $\beta$ -ketone ester or  $\beta$ -diketone groups that can react with FA, affording 1,4-DHPs with red or near-IR emission wavelengths.

Extremely simple polymers (i.e., random copolymer structure and broad polydispersity indices (PDIs)) were used; however, a biocompatible polymeric probe was developed to successfully detect endogenous FA in living systems. This result suggested that the combination of polymer chemistry and organic reactions is feasible to explore new functional polymers for bio-applications. Currently, well-defined polymers (controlled molecular weights, narrow PDIs, tunable sequences, and complex topological structures) can be rapidly prepared by modern controlled radical polymerization (CRP) such as single electron transfer-atom transfer radical polymerization (SET-ATRP), sulfur-free reversible addition-fragmentation chain transfer (RAFT) emulsion polymerization, photoinduced ATRP, and electron/energy transfer-RAFT (PET-RAFT).<sup>42-55</sup> Recent studies have reported that polymer structures (i.e., sequences and topology structures) remarkably affect the properties or functions of polymers.<sup>53, 56-60</sup> Thus, the future combination of these modern CRP techniques with the method used herein might offer new polymeric probes for the more rapid, more precise detection of FA in biological systems.

#### CONCLUSIONS

In summary, polymeric probes have been prepared for the successful detection of endogenous FA in living systems via the Hantzsch reaction. Large amounts of polymers were easily prepared, which avoided laborious multi-step syntheses of other probes. The polymer structures led to the improved reactivity and safety of the functional groups; thus, these polymers are



considerably better probes than small molecules for the detection of FA in living systems. This study highlights the combination of polymer chemistry and organic reactions to achieve new functional polymers with useful properties.

Currently, polymers have been considered to bridge laboratory organic reactions and real applications, and several organic reactions are playing new roles in polymer chemistry to achieve promising materials. Examples include click and click-inspired reactions and multicomponent reactions.<sup>61-74</sup> Thus, this first polymeric probe for the detection of FA in biological systems via the Hantzsch reaction might prompt a broad, in-depth study of organic reactions in polymer science for discovering several new functional polymers with interesting properties for applications in biological and medical areas.

## EXPERIMENTAL SECTION

### Preparation of Copolymers

$\beta$ -Ketone-ester-containing copolymers (P1, P2) were easily prepared using commercially available monomers by convenient radical polymerization. Typically, AEMA (5.4 g, 25 mmol), PEGMA (23.8 g, 25 mmol) and ABVN (248 mg, 1 mmol) were charged into a Schlenk tube with 30 mL of DMF. The Schlenk tube was sealed with a rubber septum and purged by nitrogen flow for 20 min, then kept in a 70°C oil bath for 12 h. The polymerization was quenched in an ice-water bath. Samples (~20-50  $\mu$ L) were taken for <sup>1</sup>H NMR and GPC analyses. The polymer (P1) was purified by precipitation in diethyl ether three times and then dried under vacuum to a white powder (28.4 g, ~97%).

P2 was prepared using a different AEMA/PEGMA feed ratio (1/2) by a similar procedure.

### Cell Culture

L929 cells (a fibroblast cell line from mice) were cultured at 37°C and 5% CO<sub>2</sub> in a Roswell Park Memorial Institute-1640 (RPMI-1640) medium with fetal bovine serum (FBS, 10%) and 1% penicillin and streptomycin. Culture medium was changed every other day to maintain the exponential growth of the cells.

### Experimental animals

All the experimental procedures involving zebrafish were approved by the Animal Care and Use Committee of Tsinghua University. Tuebingen zebrafish (~7 days old) were raised in an aquaculture system (25°C, 12 h/12 h (light/dark) cycle) and fed with newly paramecium three times daily at 9:00, 15:00, and 21:00. The zebrafish (~15 days old) were used for FA detection experiments.

## ASSOCIATED CONTENT

### Supporting Information

Detailed synthesis and characterization of polymers, detection of FA, cell experiments etc. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## Notes

The authors declare no competing financial interests.

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